OIPE JC141 JUL 09 2003 PATENT & TRADEMARK OFFICE

1651

Patent
Attorney's Docket No. 016800-448

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	)	
	)	
Dominique BERNARD et al.	)	Group Art Unit: 1651
	)	
Application No.: 09/884,953	)	Examiner: Jon P. Weber
	)	
Filed: June 21, 2001	)	Confirmation No.: 3212
	)	
For: ISOLATED CATHEPSIN L TYPE CYSTEINE PROTEASES AND REDUCING INTERCORNEOCYTE COHESION/PROMOTING DESQUAMATION THEREWITH	)	

AMENDMENT/REPLY TRANSMITTAL LETTER

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Enclosed is a Supplemental Response for the above-identified patent application.

[ ] A Petition for Extension of Time is also enclosed.

[ ] A Terminal Disclaimer and the [ ] $55.00 (2814) [ ] $110.00 (1814) fee due under 37 C.F.R. § 1.20(d) are also enclosed.

[X] Also enclosed is/are Annexes 1-8, Information Disclosure Statement Transmittal Letter, Information Disclosure Statement, and PTO 1449 with copies of references.

[ ] Small entity status is hereby claimed.

[ ] Applicant(s) requests continued examination under 37 C.F.R. § 1.114 and enclose the [ ] $375.00 (2801) [ ] $750.00 (1801) fee due under 37 C.F.R. § 1.17(e).

[ ] Applicant(s) requests that any previously unentered after final amendments not be entered. Continued examination is requested based on the enclosed documents identified above.

[ ] Applicant(s) previously submitted ___, on ___, for which continued examination is requested.

[ ] Applicant(s) requests suspension of action by the Office until at least ___, which does not exceed three months from the filing of this RCE, in accordance with 37 C.F.R. § 1.103(c). The required fee under 37 C.F.R. § 1.17(i) is enclosed.

[ ] A Request for Entry and Consideration of Submission under 37 C.F.R. § 1.129(a) (1809/2809) is also enclosed.

[X] No additional claim fee is required.

[ ] An additional claim fee is required, and is calculated as shown below:

AMENDED CLAIMS					
	NO. OF CLAIMS	HIGHEST NO. OF CLAIMS PREVIOUSLY PAID FOR	EXTRA CLAIMS	RATE	ADD'L FEE
Total Claims		MINUS =		× $18.00 (1202) =	
Independent Claims		MINUS =		× $84.00 (1201) =	
If Amendment adds multiple dependent claims, add $280.00 (1203)					
Total Claim Amendment Fee					
If small entity status is claimed, subtract 50% of Total Claim Amendment Fee					
TOTAL ADDITIONAL CLAIM FEE DUE FOR THIS AMENDMENT					

[ ] A total fee in the amount of $ _____ is enclosed.

[ ] Charge $_____ to Deposit Account No. 02-4800.

The Director is hereby authorized to charge any appropriate fees under 37 C.F.R. §§ 1.16, 1.17, 1.20(d) and 1.21 that may be required by this paper, and to credit any overpayment, to Deposit Account No. 02-4800. This paper is submitted in duplicate.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

Date: July 9, 2003

By: Jennifer Topmiller
Jennifer A. Topmiller, Ph.D.
Registration No. 50,435

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Alexandria, Virginia 22313-1404
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JUL 0 9 2003
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For: ISOLATED CATHEPSIN L TYPE CYSTEINE PROTEASES AND REDUCING INTERCORNEOCYTE COHESION/PROMOTING DESQUAMATION THEREWITH	)	

SUPPLEMENTAL RESPONSE

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Further to the Amendment/Response filed June 26, 2003, submitted herewith are Annexes 1-8, inadvertently omitted from the Amendment/Response filed June 26, 2003.

If there are any questions concerning this Supplemental Response or the application in general, the Examiner is respectfully requested to telephone Applicants' undersigned representative so that prosecution may be expedited.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

Date: July 9, 2003

By: Jennifer A. Topmiller
Jennifer A. Topmiller, Ph.D.
Registration No. 50,435

P.O. Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620

L'OREAL . A 97128 JP

ANNEX 1

ICN

Alphabetical List of Products

CATALOG NUMBER | | PRICE

191343 -20°C
CATHEPSIN B — 50 µg 2985.00; 100 µg 5545.00
[9047-22-7]
From Human Liver
A cysteine proteinase.
Supplied in a frozen solution of 25 mM sodium acetate buffer, pH 5.1, 0.1 mM EDTA; 0.02% sodium azide, 50% glycerol.
Purity: >95% by SDS-PAGE
MW 27,500
Ref.: Rich, D.H., et al., (1986). Biochem. J., 235, 731.

NEW 195253 -20°C
CATHEPSIN B — 10 µg 3294.50
[9047-22-7]
From Rat Liver
A cysteine proteinase.
Supplied in a frozen solution of 25 mM sodium acetate buffer, pH 5.1, 0.1 mM EDTA; 0.02% sodium azide, 50% glycerol.
MW 27 kDa

647311 0-5°C
CATHEPSIN B — 1 ml 1220.50
POLYCLONAL ANTIBODY
Anti-Human
Host: sheep
Form: liquid
Conc/Titer: 1:50-1:100
Applications: Tissue sections
Cytology Grade

NEW 194253 -20°C
CATHEPSIN B SUBSTRATE — 1 mg 127.50; 5 mg 450.00
(Z-Arg-Arg-pNA 2HCl)
Dihydrochloride
Purity: >95%
Lyophilized
MW 658.6

131161 0°C
CATHEPSIN C — 5 U 323.00; 10 U 518.00
[9032-68-2]
From Bovine Spleen
(E.C. 3.4.14.1, Dipeptidyl peptide hydrolase)
Analytical Grade
Solution in glycerol 50% (v/v)
Activity: Approx. 3 u/mg (37°C; Gly-Phe-4-nitroanilide as substrate.)

NEW 194359 -20°C
CATHEPSIN C SUBSTRATE — 1 mg 75.30; 5 mg 155.30; 25 mg 562.50
[21027-72-5]
(H-Gly-Phe-pNA HCl)
Hydrochloride
Purity: >95%
Lyophilized
Ref.: Planta, R.J. and Gruber, M.A., Anal. Biochem., 5, 360 (1963).
MW 378.9

NEW 134973 -20°C
CATHEPSIN D — 15 U 3204.00
[9025-26-7]
From Human Liver
A lysosomal aspartyl protease in mammalian cells.
Lyophilized
Activity: 330 units/mg protein.
Unit Definition: one unit is the amount of enzyme that digests acid-denatured hemoglobin-releasing peptides soluble in 10% TCA.
Purity: >98%

191344 -20°C
CATHEPSIN G — 100 µg 578.50; 500 µg 2240.00
[107200-92-0]
From Human Neutrophils
E.C.3.4.21.20.
Enzyme degrades collagen and proteoglycans, and also exhibits releasing activity of angiotensin II from angiotensin I and angiotensinogen.
Purity: >98% by SDS-PAGE
MW 23,500.

647321 0-5°C
CATHEPSIN G — 1 ml 1220.50
POLYCLONAL ANTIBODY
Anti-Human
Host: sheep
Form: liquid
Cytology Grade

NEW 134971 -20°C
CATHEPSIN G SUBSTRATE — 1 mg 75.30; 5 mg 162.70; 25 mg 562.50
[70967-97-4]
(Suc-Ala-Ala-Pro-Phe-pNA)
Purity: >95%
Lyophilized
Ref.: Nakajima, J.C., et al., Anal. Biochem., 254, 4027 (1979).
MW 624.7

NEW 134972 -20°C
CATHEPSIN H — 10 mU 2204.30
[60748-73-4]
(Cathepsin Bs; EC 3.4.22.16)
From Human Liver
Aminopeptidase by acting on substrates with free N-amino-terminal and as an endopeptidase. Involved in catabolism of lysosomal system proteins.
Activity: 200 milliunits/mg protein.
Unit Definition: one unit is the amount of enzyme that hydrolyzes 1.0 µmol of L-Arg-β-naphthylamide per minute at 40°C, pH 6.8.
Purity: >90%
Supplied in 50 mM NaOAc, pH 5.5, 1 mM EDTA.
Ref.: Taniguchi, K., et al., Brain. Res., 601, 143 (1993).
MW 28 kDa

NEW 194973 -20°C
CATHEPSIN L — 10 mU 2212.50; 100 mU 5352.50
(EC 3.4.22.15)
From Human Liver
Most powerful of the lysosomal proteinases with higher specific activity than cathepsin B and H.
Activity: 2 units/mg protein.
Unit Definition: one unit is the amount of enzyme that hydrolyzes 1.0 µmol of Z-Phe-Arg-AFC per minute at 30°C, pH 5.5.
Purity: >80%
Supplied in 20 mM NaOAc, 300 mM NaCl, pH 5.0, 1 mM EDTA.
Ref.: McDonald, J.K., et al., Biochem. Biophys. Res. Commun., 151, 827 (1988).
MW 29 kDa

Alphabetical List of Products

ICN

CATALOG NUMBER | | PRICE

873809 -20°C
CAVEOLIN — 100 µg 5310.00
MONOCLONAL ANTIBODY
(22kDa RSV-SRC Substrate, Anti-)
Clone: 2283
Isotype: purified Ig fraction from mouse ascites
Conc/Titer: 1:1,000-1:5,000
Applications: ELISA; Immunoblotting; Immunoprecipitation; Immunohistology
It has been suggested that this protein is a mediator of transformation by the tyrosine kinase class of oncogenes. For this product, a monoclonal antibody (ICN PY20) was coupled to agarose and used as an affinity chromatography medium to isolate phosphotyrosine-containing proteins from Rous sarcoma virus-transformed chick embryo fibroblasts. Balb/c mice were immunized with these PY20 proteins for production of antibodies to the 22 kDa tyrosine kinase substrate. Hybridomas were selected for reactivity against the mixture of PY-proteins by ELISA and against the 22 kDa protein by Western Blot.
The antibody is packaged at a concentration of 1 mg/ml in 50% glycerol, 5 mM Tris, pH 8.2, 25 mM NaCl, 1.5 mM NaN₃. It is easily conjugated to enzymes and fluorochromes.
Ref: 1. Glenney, J.R., Jr., J. Biol. Chem., 264, 20163-20166 (1989).
2. Glenney, J.R., Jr. and Zokas, L., J. Biol. Chem., 109, 2401-2408 (1989).

100292 0-5°C
N-CBZ-DL-ALANINE — 5 g 63.50; 25 g 269.00; 100 g 2693.50
[25807-51-2]
Crystalline
$C_{11}H_{13}NO_4$ MW 223.2

100203 0°C
N-CBZ-D-ALANINE — 5 g 107.50; 25 g 382.30; 100 g 1087.50
[4132-86-9]
Crystalline
$C_{11}H_{13}NO_4$ MW 223.2

100204 0°C
N-CBZ-L-ALANINE — 5 g 93.30; 25 g 321.30; 100 g 1053.30
[1142-20-7]
Crystalline
$C_{11}H_{13}NO_4$ MW 223.2

101198 0°C
N-α-CBZ-L-ARGININE — 5 g 99.30; 10 g 197.30; 25 g 349.30; 100 g 1068.50
[1234-35-1]
Crystalline
$C_{14}H_{20}N_4O_4$ MW 308.3

190871 0°C
N-α-CBZ-L-ARGININE — 1 g 54.30; 5 g 213.30; 10 g 357.50; 25 g 714.50
[56672-63-0]
Hydrochloride
Crystalline
$C_{14}H_{20}N_4O_4$·HCl MW 344.8

100208 0°C
N-CBZ-L-ASPARAGINE — 5 g 58.00; 10 g 168.50; 25 g 361.00; 100 g 1155.00
[2304-96-3]
Crystalline
$C_{12}H_{14}N_2O_5$ MW 266.3

101293 0°C
N-CBZ-L-ASPARAGINE-p-NITROPHENYL ESTER — 500 mg 95.50; 1 g 159.30

100210 0-5°C
N-CBZ-DL-ASPARTIC ACID — 5 g 125.; 10 g 243.; 25 g 497.
[4515-21-3]
Crystalline
$C_{12}H_{13}NO_6$ MW 267.2

100211 0-5°C
N-CBZ-L-ASPARTIC ACID — 5 g 71.; 10 g 149.; 25 g 282.; 100 g 915.
[1152-61-0]
Crystalline
$C_{12}H_{13}NO_6$ MW 267.2

100097 0°C
CBZ CHLORIDE — 50 g [illegible]; 100 g [illegible]
[501-53-1]
(Carbobenzoxy chloride; Benzyl chloroformate)
Purity: 95-98%
Reagent for preparing CBZ-amino acids. Water-white, solvent-free material. We have found other dealers offering yellowish material for higher prices.
$C_8H_7ClO_2$ MW 170.6

100215 0°C
N-CBZ-L-GLUTAMIC ACID — 25 g 132.; 100 g 435.
[1155-62-0]
Crystalline
$C_{13}H_{15}NO_6$ MW 281.3

100213 0°C
N-CBZ-L-GLUTAMINE — 5 g 115.; 25 g 353.; 100 g 1081.
[2650-64-6]
Crystalline
$C_{13}H_{16}N_2O_5$ MW 280.3

100216 RT
N-CBZ-GLYCINE — 5 g 53.; 25 g 197.; 100 g 431.; 500 g 1575.
[1138-80-3]
Crystalline
$C_{10}H_{11}NO_4$ MW 209.2

101250 0-5°C
N-CBZ-GLYCINE-p-NITROPHENYL ESTER — 1 g 71.; 5 g 235.; 25 g 393.
[1738-86-9]
Crystalline
$C_{16}H_{14}N_2O_6$ MW 330.3

100217 0°C
N-CBZ-GLYCYLGLYCINE — 5 g [illegible]; 10 g [illegible]; 25 g [illegible]; 100 g 3237.
[2566-19-0]
Crystalline
$C_{12}H_{14}N_2O_5$ MW 266.3

131253 0°C
N-CBZ-GLYCYLGLYCINE-p-NITROPHENYL ESTER — 1 g 182.; 5 g 712.; 25 g 2359.
[13574-81-7]
Crystalline
$C_{18}H_{17}N_3O_7$ MW 387.3

150872 0°C
N-CBZ-GLYCYLGLYCYL-L-ARGININE-4-METHYLCOUMARYL-7-AMIDE — 1 mg 98.; 5 mg 387.
Fluorogenic substrate for urokinase
$C_{28}H_{33}N_7O_7$ MW 579.6

100218 0°C
N-CBZ-D-HISTIDINE — 250 mg 208.; 1 g 749.
[19728-57-5]
Crystalline
$C_{14}H_{15}N_3O_4$ MW 289.3

100219 0°C
N-CBZ-L-HISTIDINE — 250 mg 57.; 1 g 159.; 5 g 733.
[14997-58-1]
Crystalline
$C_{14}H_{15}N_3O_4$ MW 289.3

100220 0°C
N-CBZ-HYDROXY-L-PROLINE — 5 g 109.; 10 g 348.; 25 g 765.; 100 g 2217.
[13504-85-3]
Crystalline
$C_{13}H_{15}NO_5$ MW 265.3

[illegible]

Compute pI/Mw

Annex 2 -

CATL_HUMAN (P07711)

DE CATHEPSIN L PRECURSOR (EC 3.4.22.15) (MAJOR EXCRETED PROTEIN) (MEP).
OS Homo sapiens (Human).

The computation has been carried out on the complete sequence.

Molecular weight: 37564.13

Theoretical pI: 5.32

ExPASy Home page	Site Map	Search ExPASy	Contact us	Proteomics tools	SWISS-PROT

Complete HCL

L'OREAL
OR 99128 JP

Compute pI/Mw

Annexe 3

CATL_HUMAN (P07711)

DE CATHEPSIN L PRECURSOR (EC 3.4.22.15) (MAJOR EXCRETED PROTEIN) (MEP).
OS Homo sapiens (Human).

The computation has been carried out on a user selected segment from position 114 to position 333 in this sequence of 333 residues.

Considered sequence fragment:

```
      61         71         81         91        101        111
      |          |          |          |          |          |
  61                                                    APRSVDW   120
 121 REKGYVTPVK NQGQCGSCWA FSATGALEGQ MFRKTGRLIS LSEQNLVDCS GPQGNEGCNG  180
 181 GLMDYAFQYV QDNGGLDSEE SYPYEATEES CKYNPKYSVA NDTGFVDIPK QEKALMKAVA  240
 241 TVGPISVAID AGHESFLFYK EGIYFEPDCS SEDMDHGVLV VGYGFESTES DNNKYWLVKN  300
 301 SWGEEWGMGG YVKMAKDRRN HCGIASAASY PTV
```

Molecular weight: 24169.75

Theoretical pI: 4.68

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part of HCL

L'Oréal
OM1128 BF

Biogenesis

Cat. No.	1911-0507
Lot No.	A960711
Quantity	1 ml

1 D MOUTONS

Annex 4

L'ORÉAL 04 93 128 JP

DISTRIBUTEUR
valbiotech
57, BOULEVARD DE LA VILLETTE 75010 PARIS
TÉL : 33 (0)1 40 03 89 14
FAX : 33 (0)1 44 52 92 69

Polyclonal antibody to

CATHEPSIN L

Host:	Sheep
Immunogen:	Highly pure Cathepsin L (EC 3.4.22.15) from human kidney
Presentation:	Liquid, Ig fraction (by selective precipitation) in PBS, pH 7.2 without preservatives
Ig concentration:	2.6 mg/ml
Assay system:	ELISA/(not tested in IHC)
Titre:	To be established in end user assay system using 1/2,500 to 1/10,000 as a guide in a simple ELISA
Cross reactivity:	Minimal cross reactivity with related serum proteins. May cross react with Cathepsin L from other species.
Storage:	Aliquot and store at 4°C, avoid contamination
Associated products:	Other conjugated antibodies to other Cathepsins, Cathepsin antigens.

02/08/94/AT

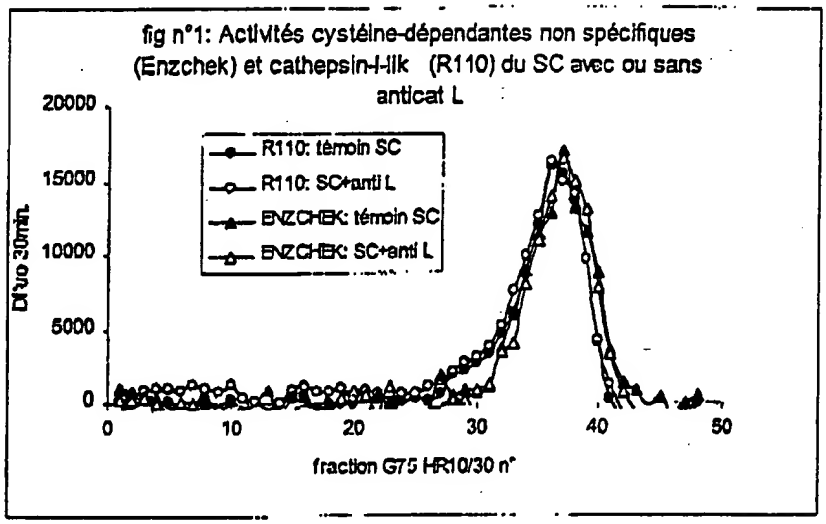
For In-Vitro Research and Manufacturing Use Only

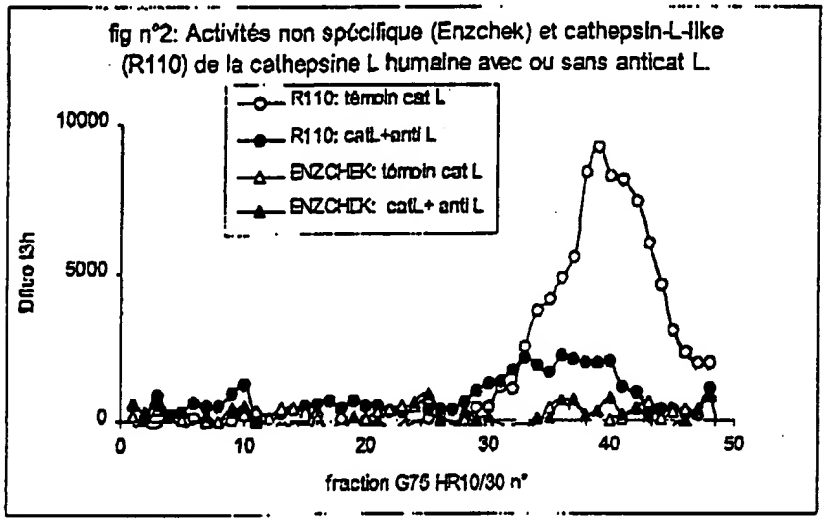
Head office: Biogenesis Ltd, 7 New Fields, Poole BH17 0NF, England, UK Telephone: (01202) 660006 Fax: (01202) 660020
US Subsidiary: Biogenesis Inc, 104 Little Mill Road, Sandown, NH 03873, USA Telephone: 603-887-4600 Fax: 603-887-4800

BLWA

IMPORTANT INFORMATION OVERLEAF

Annex 5

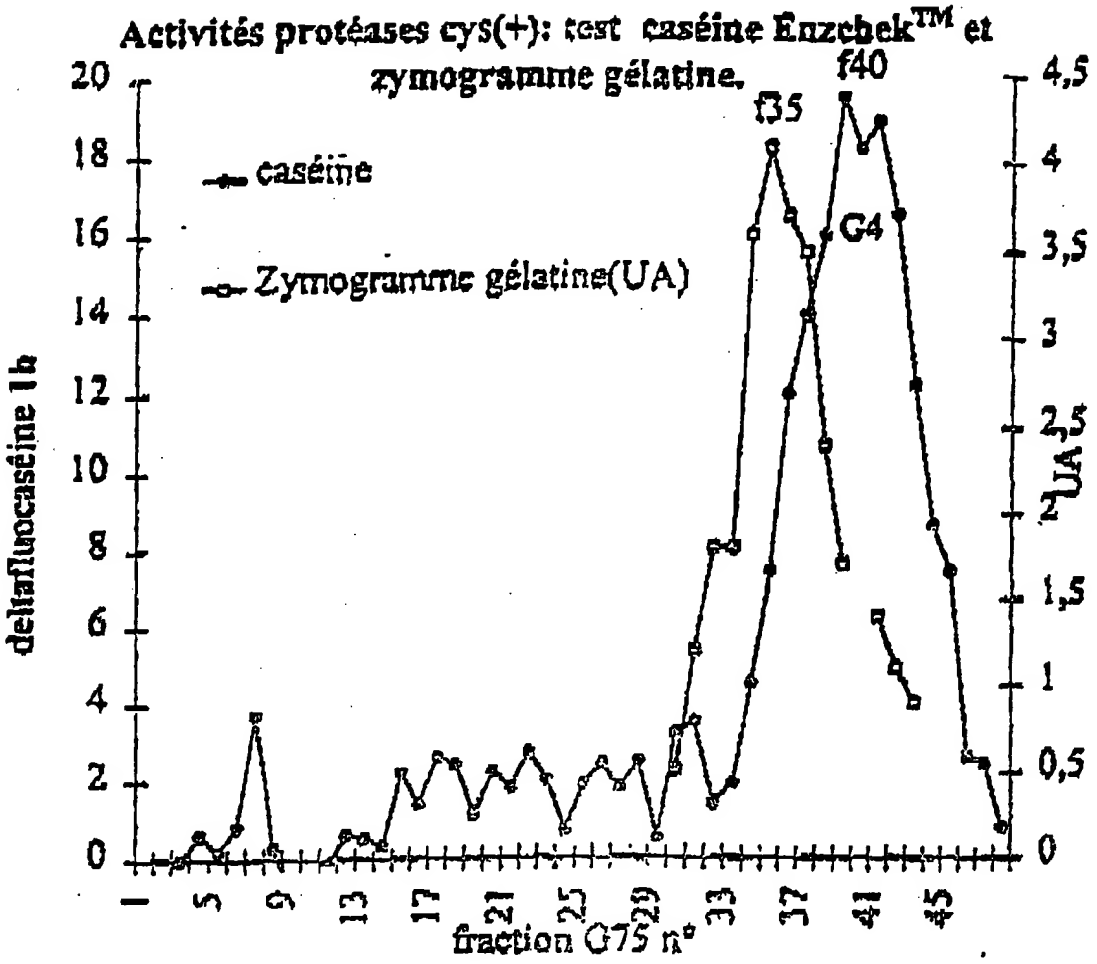




Conclusions :

Ces résultats prouvent que la protéase cystéine-dépendante du SC humain détectée à la fois par le test ENZCHEK et par le substrat peptidique Z(Phe-Arg)$_2$R110 est différente de la cathepsine L humaine car :

1) Elle n'est pas du tout immunoprécipitée par l'anticorps (fig n°1) alors que l'activité cathepsine L disparaît presque totalement des surnageants dans les mêmes conditions expérimentales (fig. n°2) ;
2) Elle a un poids moléculaire apparent plus grand que la cathepsine L comme le prouve son élution plus rapide (fig n°1/fig n°2) ;
3) Elle a une spécificité de substrat sans doute très différente de la cathepsine L car elle libère une fluorescence équivalente que ce soit à partir de la caséine du test Enzchek ou à partir du peptide marqué à la Rhodamine 110 (fig. n°1) alors que l'activité cathepsine L n'est détectable dans nos conditions expérimentales que sur le substrat peptidique (fig n°2).



Annex 6

G_4 = our peptide.

L'ORÉAL ON VOUS DIT.

FAX émis par: +33 01 47 56 73 88 L OREAL le 05/01/00 14:06 A4 NORM Pg: 11/15

ANNEX 1

Biochem. J. (1988) 253, 303–306 (Printed in Great Britain)

Isolation and sequence of a cDNA for human pro-(cathepsin L)

Susannah GAL* and Michael M. GOTTESMAN†
Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Building 37, Room 2E18, Bethesda, MD 20892, U.S.A.

The major excreted protein (MEP) of malignantly transformed mouse fibroblasts is the precursor to an acid proteinase with enzymic specificity similar to that of human cathepsin L. By cross-hybridization with a mouse MEP sequence, cDNA clones of the human form of MEP in an SV40 expression vector were isolated. A 1.6 kb cDNA showed 70% deduced amino acid sequence identity with mouse MEP. The deduced amino acid sequence of the cloned human MEP was the same, except for two amino acids, as the *N*-terminal sequence of mature human cathepsin L, thereby establishing that human MEP is human pro-(cathepsin L). Use of this human pro-(cathepsin L) cDNA clone allowed the detection of a 1.6–1.8 kb pro-(cathepsin L) mRNA in human cells which was not detected with a mouse pro-(cathepsin L) probe.

INTRODUCTION

Mouse fibroblasts which are malignantly transformed or stimulated by growth factors or tumour promoters synthesize and secrete increased amounts of a 39 kDa glycoprotein with acid-proteinase activity (Gottesman, 1978; Gottesman & Sobel, 1980; Doherty *et al.*, 1985; Rabin *et al.*, 1986; Frick *et al.*, 1985; Nilson-Hamilton *et al.*, 1981; Gal & Gottesman, 1986*a*). This protein, termed 'MEP' (major excreted protein), is the precursor to two lower-*M*, lysosomal proteins of 29 kDa and 21 kDa (Gal *et al.*, 1985), and contains the lysosomal recognition marker mannose 6-phosphate (Sahagian & Gottesman, 1982). Mouse MEP and human cathepsin L (Mason *et al.*, 1985) share amino acid bond-cleavage specificities, catalytic constants and inhibitor susceptibilities (Gal & Gottesman, 1986*b*; Mason *et al.*, 1987). Comparison of the deduced amino acid sequences of mouse MEP, or a related protein called 'mouse cysteine proteinase', with a partial amino acid sequence for human cathepsin L, indicates strong similarity between these two sequences, but because of the species differences, does not prove identity (Troen *et al.*, 1987; Portnoy *et al.*, 1986; Denhardt *et al.*, 1986; Mason *et al.*, 1986).

In the present paper, we report the isolation and characterization of a full-length human MEP cDNA clone, isolated by cross-hybridization with a mouse MEP cDNA clone. The deduced amino acid sequence from this clone was identical, except for two amino acids, with that of the human cathepsin L sequence, proving that MEP is cathepsin L. This human cathepsin L cDNA can be used as a probe to determine the expression of cathepsin L mRNA in human cell lines and tissues.

MATERIALS AND METHODS

Isolation of a human cDNA clone for cathepsin L

The Okayama–Berg (1983) cDNA expression library termed 'GM637', made from mRNA isolated from SV40-virus-transformed human fibroblasts, was a gift from Dr. Hiroto Okayama (National Institute of Mental Health, National Institutes of Health, Bethesda, MD, U.S.A.). In all, 100000 clones were screened on 20 filters by the technique of Troen (1987) for colony hybridization, using the 800-bp restriction-endonuclease-*Eco*RI fragment of mouse MEP (Troen *et al.*, 1987) labelled by nick translation (Lofstrand Laboratories, Gaithersburg, MD, U.S.A.). The filters were washed under moderate stringency conditions: twice with 2×SSC (1×SSC = 0.15 M-NaCl/0.015 M-sodium citrate)/1% SDS and twice with 0.8×SSC/1% SDS at 60 °C, and three positive clones were obtained. The clone designated 'pHu-16' contained the largest insert (1.6 kb) and was used for all subsequent studies.

Sequencing and blotting

Sequencing was done using the Sanger *et al.* 1977) dideoxy method with the Promega sequencing kit (Promega Biotec, Madison, WI, U.S.A.) and deoxyadenosine [α-^{35}S]thiotriphosphate at a specific radioactivity of 500 Ci/mmol (NEN, Boston, MA, U.S.A.). pHu-16 was sequenced by using a combination of two techniques: (1) subcloning regions of pHu-16 DNA into Promega vectors pGEM-3 and pGEM-4 with Sp6 and T7 primers (Promega), and (2) direct sequencing using 50 ng of synthetic oligonucleotides (Applied Biosystems) as primers and the entire pHu-16 cDNA as template.

Detection of human procathepsin L RNA

RNA was isolated from tissue-culture cells as previously described (Chirgwin *et al.*, 1979; Maniatis *et al.*, 1982), and Northern blots were as described by Shen *et al.* (1986). Northern blots were probed with nick-translated *Eco*RI fragments from pHu-16 cDNA or pMMEP-14 cDNA (Troen *et al.*, 1987, 1988).

RESULTS AND DISCUSSION

Isolation and sequence of a human cathepsin L cDNA

We selected the human MEP clone by screening a cDNA library made from SV40-virus-transformed

Abbreviations used: MEP, major excreted protein.

* Present address: Friedrich-Miescher-Institut, P.O. Box 2543, CH-4002 Basel, Switzerland.
† To whom correspondence and reprint requests should be addressed.

FAX émis par: +33 01 47 56 73 88 L OREAL le 05/01/00 14:06 A4 NORM Pg: 12/15

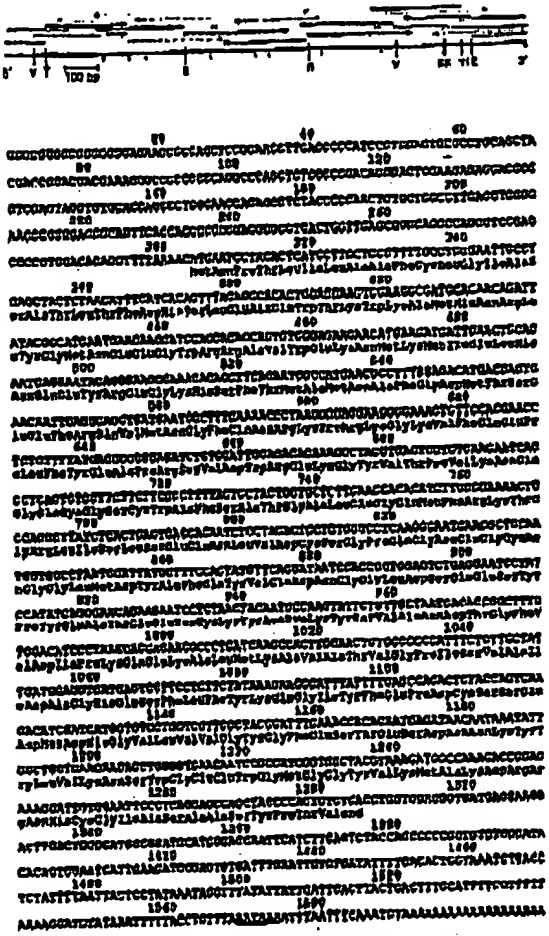


Fig. 1. (*a*) Sequencing strategy for pHu-16 and (*b*) the entire sequence of pHu-16 with the longest open reading frame translated underneath beginning at nucleotide 305 and terminating at nucleotide 1304

(*a*) Bold lines are sequences determined using subcloned fragments in pGEM3 or pGEM4 primed with Sp6 and T7 oligonucleotides. The dotted lines indicate sequences derived from the entire pHu-16 plasmid using synthetic oligonucleotides (*) as primers. The orientation of the clone is shown along with some of the restriction sites; V = *Pvu*II, T = *Taq*I, E = *Eco*RI, R = *Rsa*I, S = *Sph*I. (*b*) The poly(A) addition signal is underlined.

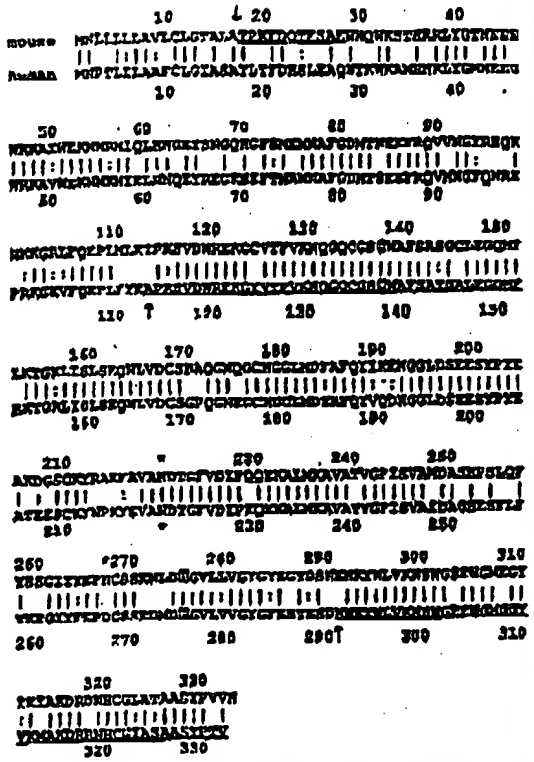


Fig. 2. Homology of mouse and human MEP sequences

Complete amino acid sequences deduced for pHu-16 (human MEP) and pMMEP-14 [mouse MEP (Troen *et al.*, 1987)] are shown. The underlined sequences of human MEP correspond to protein sequences of human cathepsin L obtained by Mason *et al.* (1986). The underlined sequence of mouse MEP corresponds to the *N*-terminal sequence of secreted MEP purified from mouse tissue-culture medium (Troen *et al.*, 1987). Arrows indicate where processing occurs, and asterisks indicate potential *N*-glycosylation sites. The proposed active-site amino acids based on papain, Cys-138 and His-276, are shown by C and H respectively. Homology is indicated as exact matches (|) and conservative substitutions (:).

human fibroblasts at low stringency using an 800-bp *Eco*RI fragment from a mouse MEP cDNA clone (see the Materials and methods section). One clone, termed 'pHu-16', contained a 1600-bp cDNA insert. The restriction map, sequencing strategy, complete nucleotide sequence and deduced amino acid sequence for pHu-16 are shown in Fig. 1.

The sequence of the human MEP cDNA revealed a single long open reading frame coding for a protein of M_r 37522. The deduced amino acid sequence was compared with the mouse MEP sequence (Fig. 2) and was found to be 70% identical over the entire sequence, with some regions having 90% identity (e.g. between amino acids 115 and 168 and between amino acids 217 and 249). Over 98% sequence identity was found between the *N*-terminal amino acid sequence of mature human cathepsin L (Mason *et al.*, 1986) and human MEP (shown as the underlined sequence in Fig. 2). The two amino acids which are different from our predicted sequence and that published by Mason *et al.* (Pro for Glu at position 148 and Tyr for Arg at position 153) may be due to problems with the amino acid analysis or to polymorphic differences in the proteins isolated from two different human sources. These results show that MEP is pro-(cathepsin L). Similar conclusions have been reached by Joseph *et al.* (1987), who reported the partial sequence of the human analogue of a *ras*-oncogene-induced protein which appears to be identical with MEP/cathepsin L.

FAX émis par: +33 01 47 56 73 88 L OREAL le 05/01/00 14:06 A4 NORM Pg: 13/15

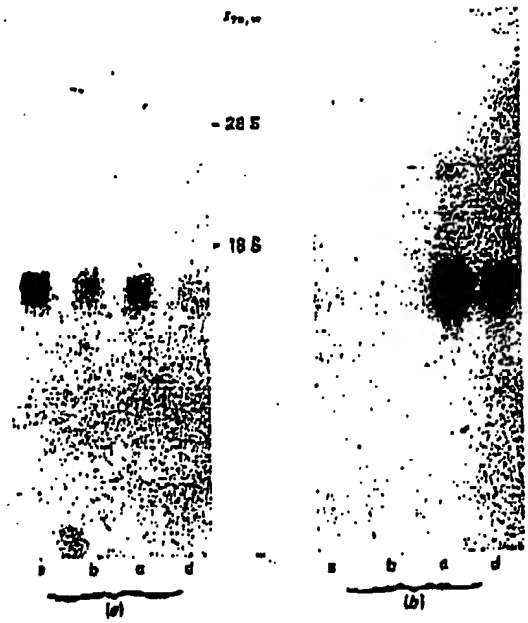


Fig. 3. Northern blots of RNA from tissue-culture cells and human tissues

(*a*) and (*b*), Northern blots of 10 μg of total RNA from tissue-culture cells using two different probes. The sources of RNA were human KB cells (a), multidrug-resistant KB cells (b), mouse NIH 3T3 cells (c) and multidrug-resistant NIH 3T3 cells (d) as previously described (Shen *et al.*, 1986). In (*a*) hybridization was with the human pHu-16 *Eco*RI fragment and in (*b*) hybridization was with the *Eco*RI fragment from the mouse MEP clone pMMEP-14 (Troen *et al.*, 1987).

As for mouse MEP and mouse cysteine proteinase (Troen *et al.*, 1987; Portnoy *et al.*, 1986; Denhardt *et al.*, 1986), significant homologies were also observed when the human MEP sequence was compared with those of other cysteine proteinases, including cathepsin B, cathepsin H, actinidin and papain. Mouse and human forms of MEP show nearly identical sequences around amino acids Cys-138 and His-276 (Fig. 2), which are putative active sites on the basis of similarities to papain (Kamphuis *et al.*, 1985). Comparison of our sequence with those of several other human lysosomal proteins shows no obvious conserved signals for the lysosomal-protein-specific transfer of UDP-*N*-acetylglucosamine to the high-mannose chain to yield the lysosomal mannose 6-phosphate marker.

The *N*-terminal sequence of secreted mouse MEP indicates where pre-MEP is processed to yield MEP (Troen *et al.*, 1987). The deduced amino acid sequence of human prepro-(cathepsin L) also has the canonical Ala-Xaa-Ala sequence which precedes the leader-sequence cleavage site (Perlman & Halvorson, 1983) (Fig. 2). The positions of the *N*-terminal peptides of mature human cathepsin L within the deduced amino acid sequence of human MEP indicate the sites at which this precursor to human cathepsin L is processed to the lower M_r mature forms.

Because the full-length pro-(cathepsin L) cDNA which we isolated is cloned downstream from the SV40 promoter (Okayama & Berg, 1983), it is possible to express this cDNA in transfected cells. Recent experiments indicate that the human pro-(cathepsin L) cDNA is expressed in mouse NIH 3T3 cells as a 42 kDa protein that is secreted when it is overproduced (Kane *et al.*, 1988).

Use of the human procathepsin L cDNA to detect a procathepsin L mRNA in human cells

Fig. 3 shows the results of Northern blots with human KB (HeLa) cell and NIH 3T3 mouse-cell RNA probed with the *Eco*RI 800-bp fragment from the mouse and human MEP cDNA clones. The human probe recognized a 1.6–1.8-kb pro-(cathepsin L) message in human KB cells which migrated just below the 18 S RNA marker (Fig. 3*a*) and appeared to be the same size as MEP mRNA from mouse cells (Fig. 3*b*). At high stringency, the mouse probe recognized a 1.6–1.8-kb RNA in mouse cells but no message in human cells (Fig. 3*b*). These data indicate that the human pro-(cathepsin L) cDNA described here (pHu-16) can be used to detect a human pro-(cathepsin L) mRNA, and that there is only one major pro-(cathepsin L) mRNA in cultured human KB cells. Preliminary results indicate that all normal human tissues tested express this pro-(cathepsin L) mRNA (results not shown). The availability of a full-length cDNA probe for human pro-(cathepsin L) will make it possible to assess relative levels of pro-(cathepsin L) mRNA in different tissues and in tumours.

We thank Dr. D.-w. Shen for RNA samples, Dr. H. Okayama for the human cDNA library, Dr. B. Troen, Mr. S. Smith and Dr. S. Kane for helpful discussions, Mr. S. Neal for photographic assistance, and Ms. J. Sharrar for secretarial help.

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Annex 8

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January 5, 2000

Your Ref: OA97128/JP/BN:CG
Our Ref: JP1060LOR

Re: Japanese Patent Application No. Hei 10-244055
"Polypeptide isolated from the epidermis and its use"

Dear Mr. Galup:

Thank you for the front page of JP-A-H06-192124 (Toray). The following is an English translation of the paragraphs [0012] and [0013].

[0012]
Human cathepsin L is already known as one type of thiol protease (Mason et al., Biochemical Journal, 240, 373-377, 1986), and its ability of elastin degradation (Mason et al., Biochemical Journal, 233, 925-927, 1986) and an action of inactivating α1-protease inhibitor (Johnson et al., Journal of Biological Chemistry, 261, 14748-14751, 1986) have been reported.
[0013]
The prepro form of human cathepsin L consists of 333 amino acid residues (molecular weight of 38000), and the precursor (pro form) is the one in which N-terminal 1-17 residues are deleted. Namely, it comprises 18-333 residues (316 amino acid residues with molecular weight of 36000), and N-terminal amino acid sequence is as shown in Seq ID No: 1 of the sequencing list (Joseph et al., J. Clinic. Investigation, 81, 1621-1629, 1988). Among these, 18-113 residues are called activation peptide, 114-288 residues are a cathepsin H heavy chain, and 292-333 residues are a cathepsin L chain.

According to the paragraph [0015], the cathepsin L of the present invention includes precursor (18-333 residues), mature form (114-333 residues), H-chain (114-288 residues), L-chain (292-333 residues), and the precursor is preferable. Thus, the authors preferably use the procathepsin L. With respect to Mason et al., Biochemical Journal, 240, 373-377, 1986 or Mason et al., Biochemical Journal, 233, 925-927, 1986 or Johnson et al., Journal of Biological Chemistry, 261, 14748-14751, 1986, it appears from the context of the above two paragraphs that they describe human cathepsin L or its prepro form or pro form or the like which are mentioned in the paragraph [0013]. Therefore, we presume that the human cathepsin L described in these documents are the same as those used by the authors.

We hope we have answered your question sufficiently, but should you have any questions, please do not hesitate to contact us.

Very truly yours,

Yoshinori Kobayashi